General Project Outline:

I. Characterize FAK -/- and FAK +/+ cells (partially complete)

Hypothesis: Loss of FAK in -/- will lead to loss (? or gain) of certain cellular functions important for osteoblast differentiation and/or bone formation, and alter known osteogenic responses to dynamic fluid shear stress (oscillatory flow).

A. Ca^{2+} response
B. COX-2, PGE2
C. MAPKs
D. Mineralization potential (ALP, etc.)
E. General cell morphology/growth

II. Re-expression of FAK and FAK mutants (Y397F, and Y925F) in FAK -/- cells (partially complete)

Hypothesis: Re-expression of wtFAK in FAK -/- cells should lead to a rescue of FAK +/+ characteristics (see attached Schlaepfer, et al. paper Fig 10). Expression of FAK mutants (Y397F, and Y925F) should lead to a partial rescue of FAK +/+ characteristics.

A. Develop expression vector (complete)
B. Transfect FAK -/- cells, grow under Hygromycin B selection pressure
FAK -/- clone 1Df based cells (complete)
FAK -/- clone 1E6 based cells (partially complete – see subcloning outline)
C. Subclone transfected cells (next step – see subcloning outline and protocol)

III. Characterize new FAK -/- cells re-expressing FAK construct

This section is similar to section I. These analyses should reveal which functions are dependent on wtFAK, FAK tyrosine 397 and tyrosine 925.

Background of FAK -/-; +/+ cells:

The FAK -/- and +/+ calvarial osteoblasts (see the grant p.g. 45-46, and the Jae-Boem Kim paper attached) were provided by Jae-Boem Kim:

FAK -/- cells:

1Df

- Stably transfected (pcDNA 3.1 Hygro + w/ vector only, wtFAK, FAK Y397F, and FAK Y925F) and grew under Hygromycin B selection pressure (350 ug/ml)
- Ready for subcloning

1E6

- Transfected but grown for only 16 days
- Grow under Hygromycin B selection pressure before subcloning

FAK +/+ cells:

1D5

1E11?

The cells were originally used by Chi Hyun Kim (former lab member) to provide preliminary data for the grant. Chi Hyun froze "seed" vials of these cells (see the liquid nitrogen log book in lab, I don't know the rack and box numbers – these cells should probably be used only to grow and freeze more "stock" cells for general use). In order to further characterize these cells I grew and froze FAK -/- clone 1Df and FAK +/+ clone 1D5 (liquid nitrogen rack 4 box B, white and red cap cryo vials). FAK -/- clone 1Df cells were transfected (pcDNA 3.1 Hygro + w/ vector only, wtFAK, FAK Y397F, and FAK Y925F) and grown under

Hygromycin B selection pressure (350 ug/ml) for approximately one month (frozen in liquid nitrogen, rack 4 box B – green, yellow, violet and orange cap colors). These cells should be stably transfected and ready for subcloning (see subcloning outline). FAK -/- clone 1E6 cells were also transfected, but only grown under selection pressure for ~16 days before being frozen (rack 4 box B – pink, gray, blue, and tan caps). These cells still need to be grown under hygromycin B selection pressure before subcloning (see subcloning outline).

Background of FAK plasmids:

The original FAK expression plasmids* were a generous gift (?) from David D. Schlaepfer (Scripps Institute). Since the FAK -/- cells already contained neomycin resistance (the selection antibiotic of the pcDNA 3.0 plasmid), the FAK genes were subcloned into pcDNA 3.1 Hygro + (Invitrogen cat# V870-20) using the Kpn I and Xba I restriction enzyme sites. All DNA from the process is currently stored in the -20^{o}C freezer in lab (see boxes labeled "Do No Discard" w/ initials JJR).

* pcDNA 3.0 (http://www.addgene.org/pgvec1?f=c&cmd=showvecinfo&vectorid=5592&pf=true) containing c-terminus 3X HA-tagged wtFAK, FAK Y397F, and FAK Y925F inserted between the Kpn I and Xba I RE sites

To do:
FAK-/- 1Df – ready for subcloning
FAK +/+ 1D5
Perform initial experiments using 1Df and 1D5 cells: Proliferation, Cox-2 (mRNA, protein), PGE-2 release